Supporting Information

Pulsed Magnetic Field Improves the Transport of Iron Oxide Nanoparticles through Cell Barriers

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Supplemental experimental methods and results

1. Physicochemical characterizations of Hep-MNPs preparation. Hydrodynamic sizes and zeta potential values of MNPs showed no significant changes during the incubation time (0 or 5 h) in water or in buffer containing 10 % fetal bovine serum (FBS) (Figure S1). Zeta potential measurements showed the changes in the surface charge of MNPs in the serum-containing buffer, relative to the water (-36 (± 1.1) mV in water; -14.2 (± 2.3) mV in buffer with serum). This might be because of serum protein adsorptions on the nanoparticles.[1,2] However, the presence of serum in the buffer during the 90 min transport experiments may not affect the stability of the particles since little changes are shown in the size and also in zeta potential during the incubation with 10 % FBS.

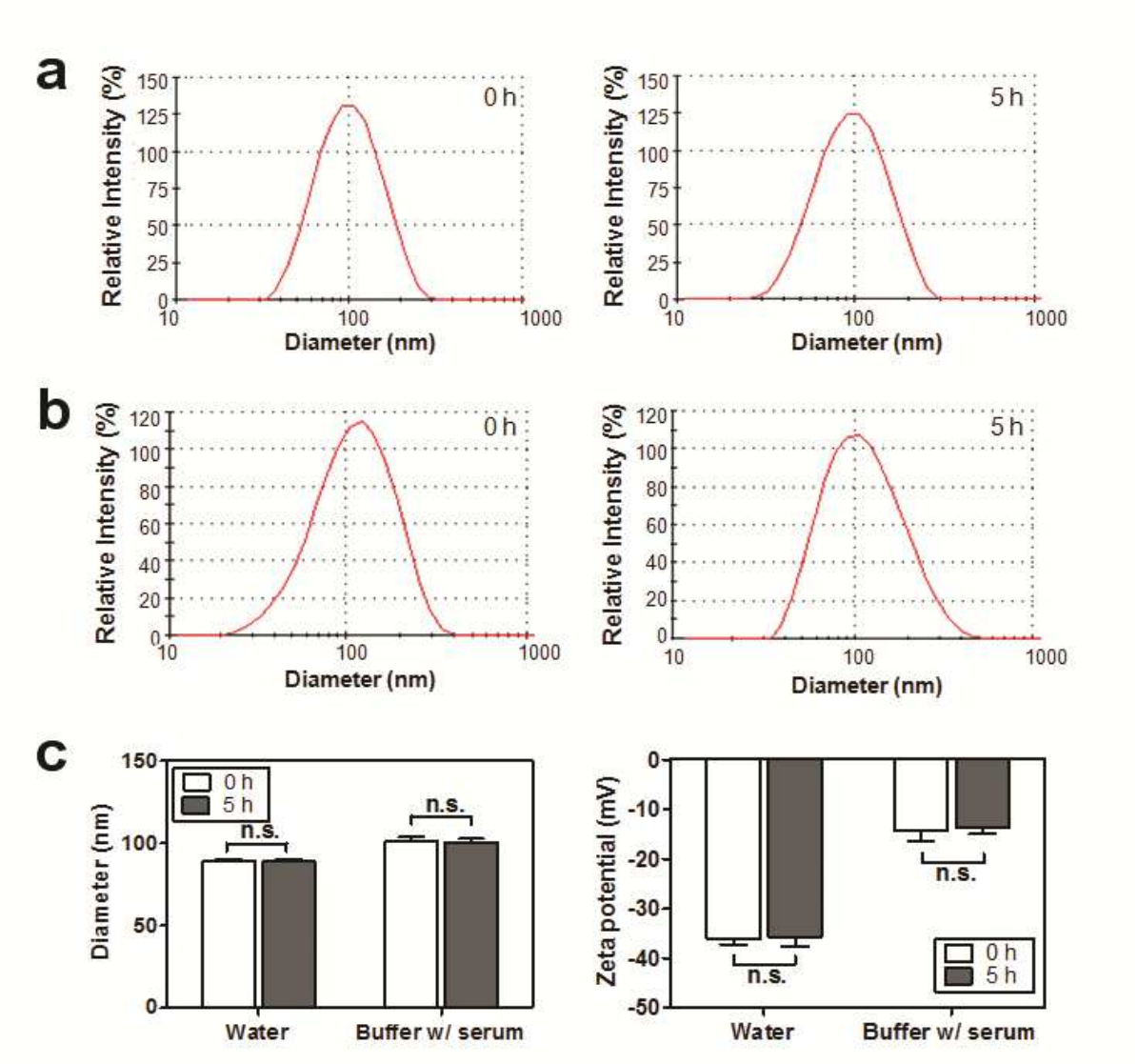

Figure S1. Stability of Hep-MNPs in physiological buffers. Size distribution profiles of MNPs in (a) water or (b) HBSS buffer with 10 % FBS after incubation at 37°C for 0 and 5 h. (c) Size (nm) and Zeta potential (mV) measurements (mean ± SD, N = 3) of MNPs in water or HBSS with 10 % FBS after incubation at 37°C for 0 and 5 h.

2. A Constant Magnetic Field Visibly Promoted Accumulation of MNPs on Cell Monolayers. After transport studies under a constant magnetic field at different initial MNPs

concentrations, the inserts were examined under the bright field microscope. As shown in Figure S2a, particle aggregates accumulated on the cell monolayers were visible as brown patches and the accumulations of particle aggregates appeared greater as the concentration of MNP was increased. To confirm this observation, the microscopic images were subjected to quantitative analyses. The area covered by brown clusters of particle aggregates on the surface of cell monolayers was measured (Figure S2b).

As previously reported,[3] an external magnetic field promoted mass transport of MNPs across cell monolayers with transport kinetics of particles dependent on the initial particle concentrations. Paradoxically, mass transport rates decreased as MNP concentration in the donor compartment was increased. In the absence of magnetic field, there was no significant difference in apical to basolateral transport of particles, in various initial donor concentrations of MNPs (Figure S3). Under constant magnetic field conditions at high MNP concentration (0.412 mg Fe/ml), the rate of transport into the basolateral compartment over time (0.63 ng Fe/sec (± 0.05)) was less than that at the lower MNP concentration (0.258 mg Fe/ml) (1.15 ng Fe/sec (± 0.11)).

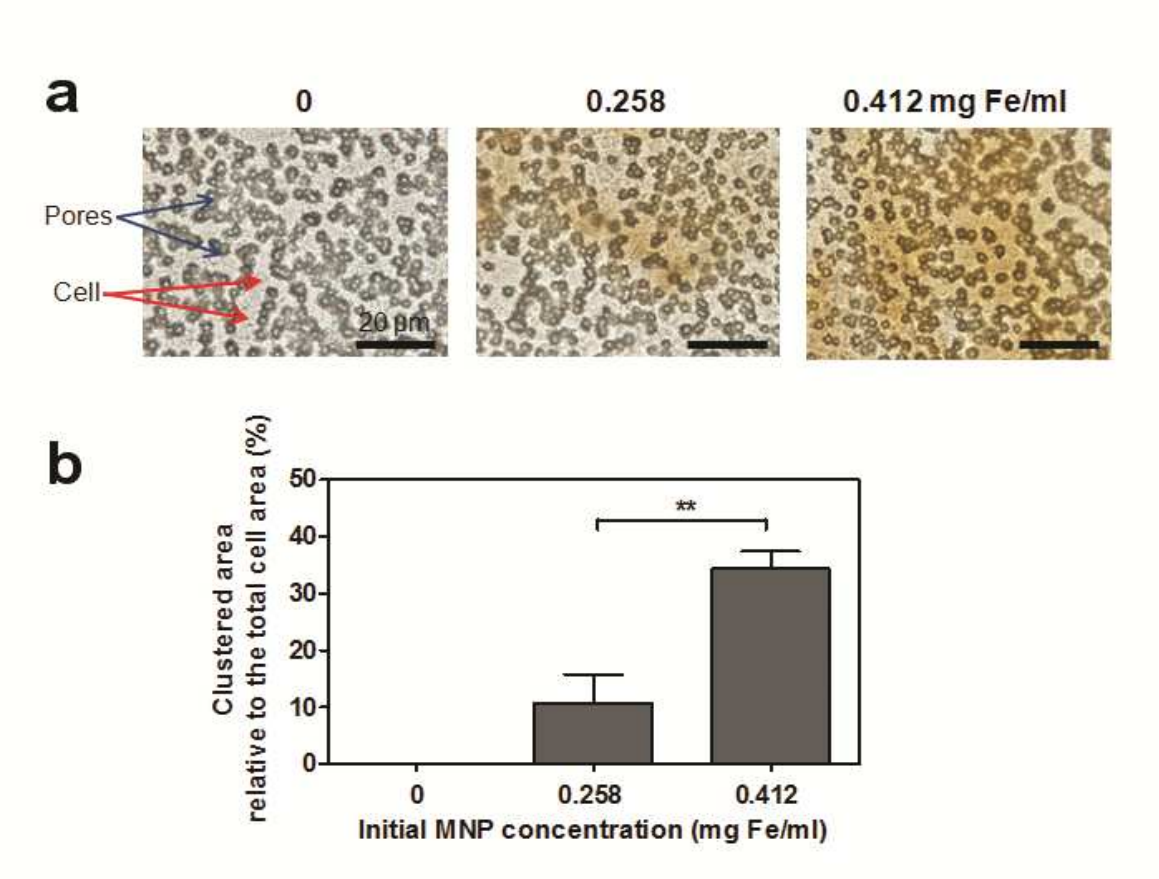

Figure S2. Microscopic images revealed visible, brown MNP aggregates covering the apical surface of cell monolayers after transport studies. (a) After a 90 min transport experiment, the cells on the insert were examined under bright field optics of Olympus BX-51 upright light microscope (100 × objectives). MNP aggregates covering the surface of the cell monolayer at 0.258 and 0.412 mg Fe/ml were visible as brown patches. No such brown patches were visible before the experiments, or in negative control inserts without MNPs. Pores on the PET membrane of inserts (pore size: 3 µm) and MDCK cells are indicated in the images with the blue and red arrows in the bright field image of the negative control. Scale bar is 20 µm. (b) Area covered by brown particle aggregates relative to the cell monolayer area (%) at the end of a 90 min transport experiment was compared for different initial apical concentrations of MNP suspension (N = 5) by unpaired t-test ($\alpha = 0.05$).

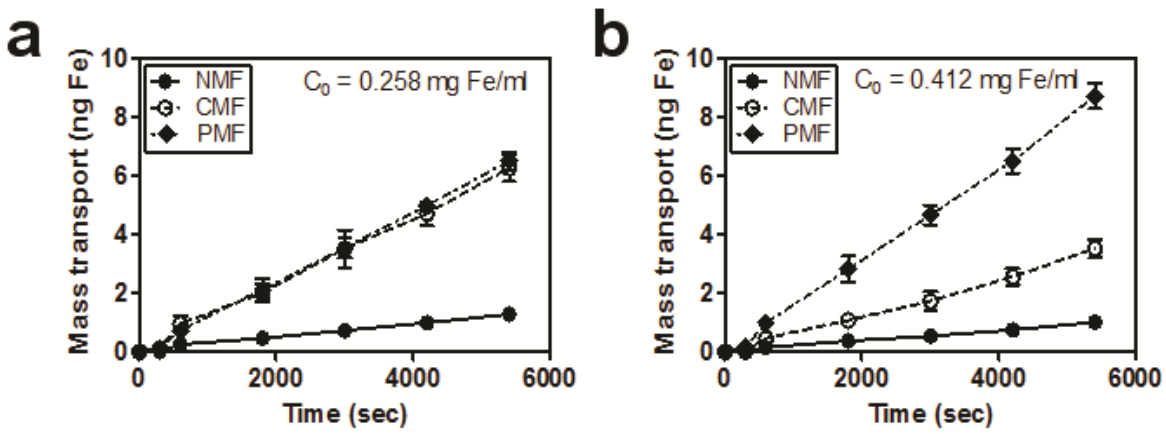

Figure S3. Mass transport of MNPs across MDCK cells in Transwell™ insert was assessed as a function of time for different initial MNP concentration in the presence or absence of the magnetic field (NMF means "no magnet", CMF "constant magnetic field", and PMF "pulsed magnetic field"; N = 3) ((a) 0.258 mg Fe/ml; (b) 0.412 mg Fe/ml).

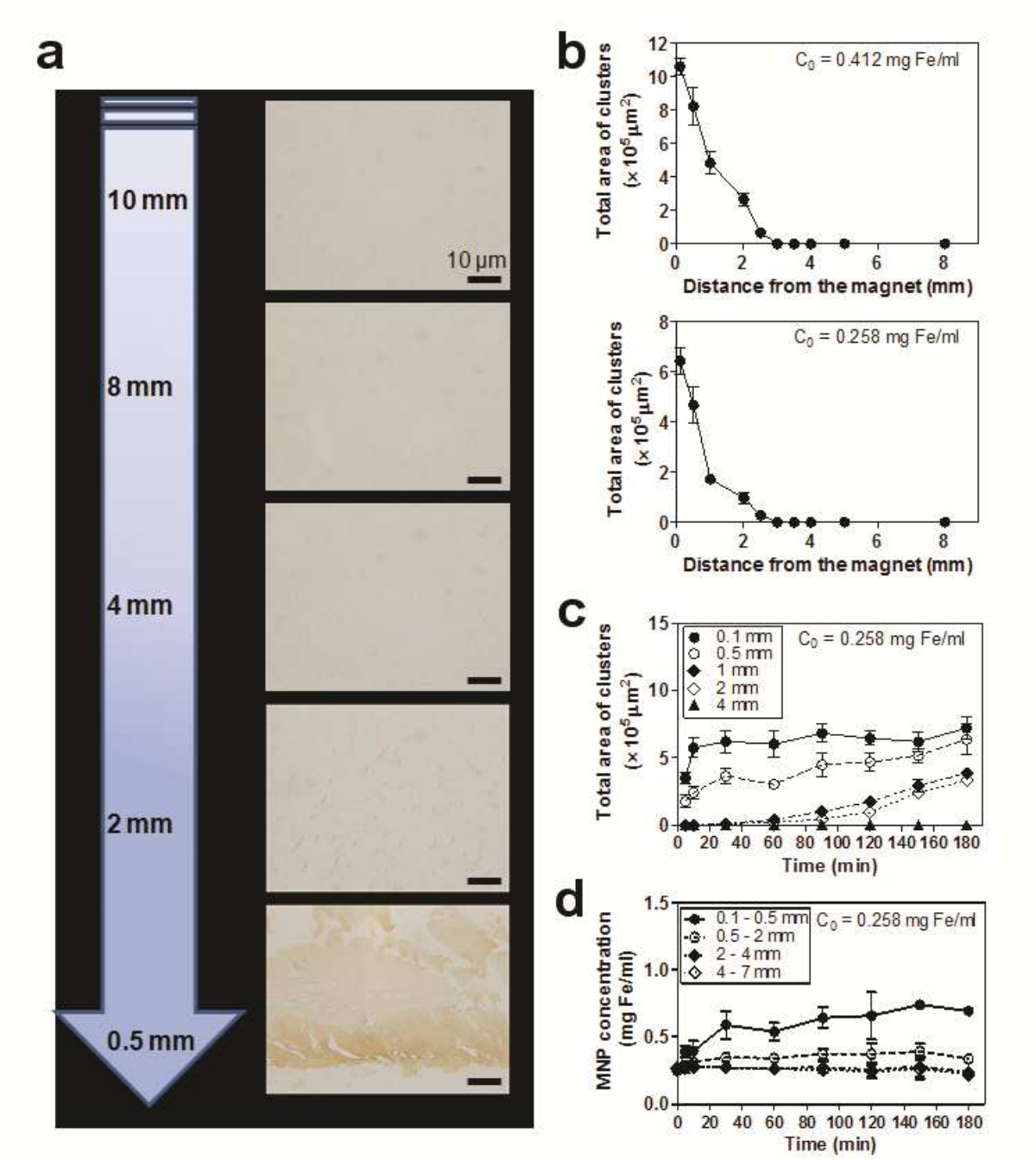

Figure S4. Microscopic examination of magnetically-induced aggregates of MNPs in suspension according to the distance from the magnet. (a) MNPs within 2 mm from the magnet showed apparent aggregates with the sizes detectable by at 1000 × magnification with an Olympus BX-51 upright light microscope (scale bar = 10 μm). Bright field images of MNPs at high

concentration (0.412 mg Fe/ml) at different distances from the magnet surface, at 3 h. (b) Total sizes (area; μm^2) of clusters of particle aggregates measured at 3 h is displayed according to the distance from the magnet between 0.1 and 8 mm for different initial MNP concentrations (0.412 or 0.258 mg Fe/ml). At lower initial MNP concentration (0.258 mg Fe/ml), (c) Total sizes (area; μm^2) of clusters of particle aggregates measured from the bright field images of particle suspension within 4 mm (0.1, 0.5, 1, 2, and 4 mm) from the magnet is displayed as a function of time (5-180 min) under the magnetic field. (d) MNP concentration changes at each segment in the tube (0.1-0.5, 0.5-2, 2-4, and 4-7 mm from the magnet) are plotted as a function of time (5–180 min) under the external magnetic field (0.258 mg Fe/ml).

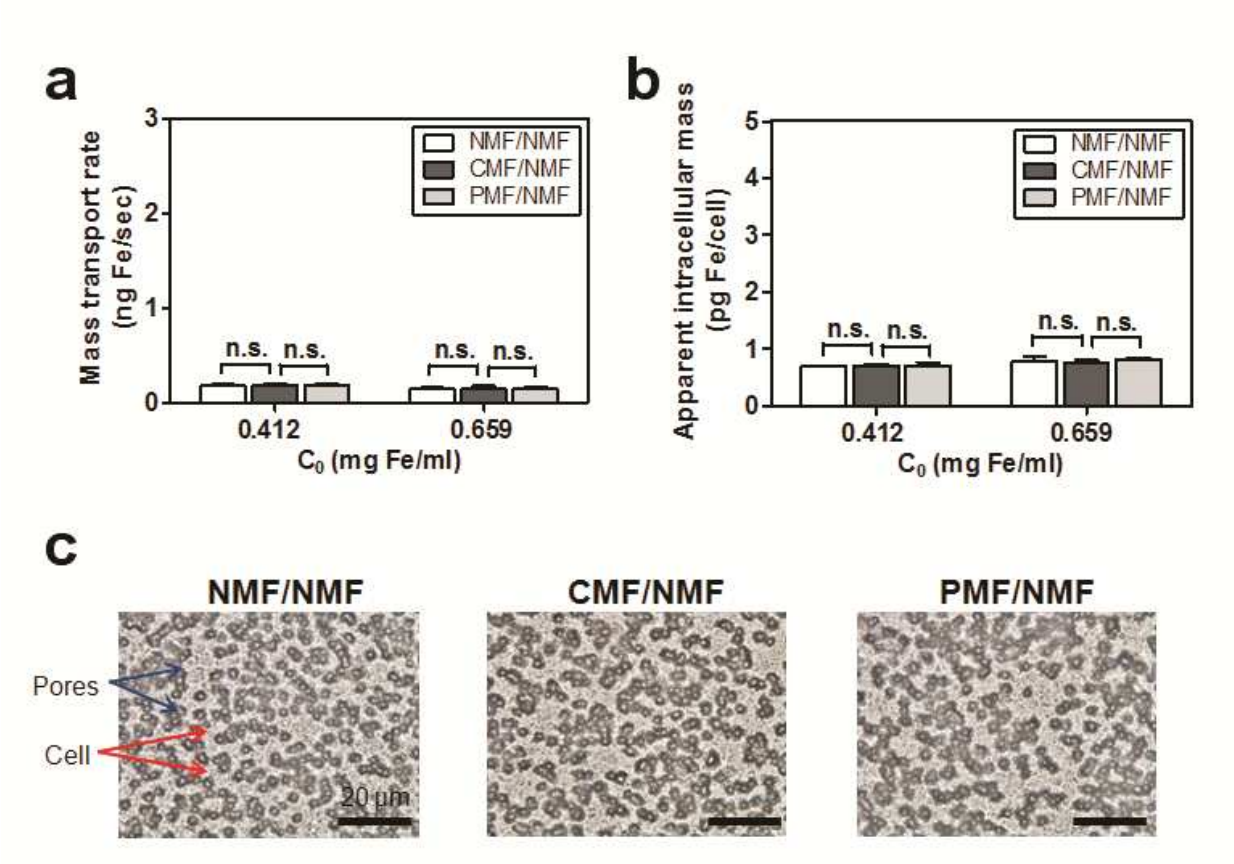

Figure S5. Mass transport of MNPs across MDCK cell monolayers was not affected by pre-exposure of MNPs to various magnetic field conditions. NMF/NMF corresponds to "the cell experiments under no magnetic field (NMF) with MNPs pretreated with no magnetic field (NMF)"; CMF/NMF means "the experiments with MNPs pretreated with a constant magnetic field (CMF)"; and, PMF/NMF is "the experiments with MNPs pretreated with a pulsed magnetic field (PMF)" (N = 3). (a) Mass transport rates of pretreated MNPs and (b) apparent intracellular masses of MNPs per cell, after a 90 min transport, for different initial MNP concentrations (C_0: 0.412 or 0.659 mg Fe/ml) under no magnetic field conditions. (c) Microscopic images of cells after transport studies in the absence of magnetic field with MNPs pretreated with different magnetic fields. Scale bars (20 μm) are presented in the images. For statistical analysis, one-way ANOVA test was used with Tukey's multiple comparison tests ($\alpha = 0.05$).

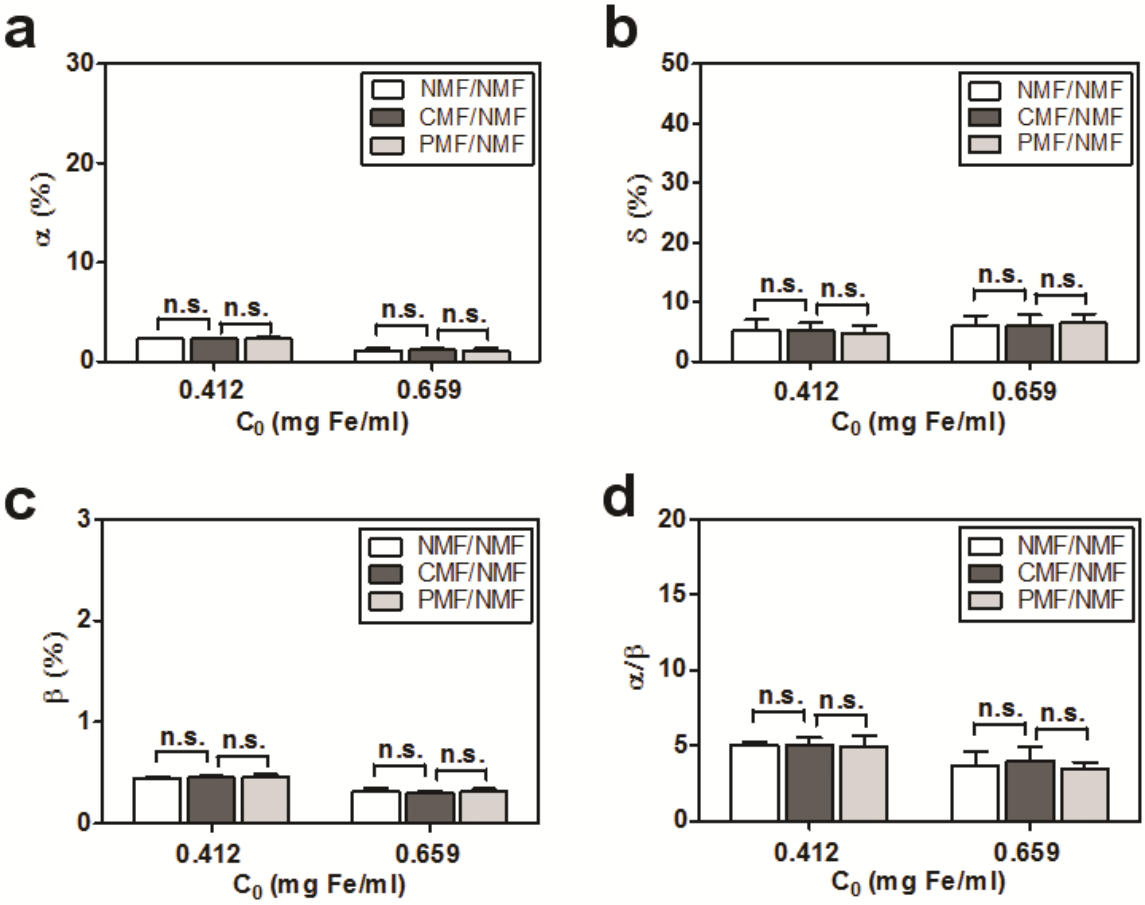

Figure S6. Mass balance analysis after transport experiments in the absence of magnet with MNPs pre-exposed to different magnetic field conditions. Transport experiments were performed at 0.412 or 0.659 mg Fe/ml (C_0) and data were subjected to mass balance analysis (equations 1-5). (a) Apical-to-basolateral transported fraction of MNPs, α (%); (b) fraction of particles bound to the cell surface, δ (%); (c) fraction of particles inside the cells, β (%). (d) Ratio (α/β) is depicted at 0.412 or 0.659 mg Fe/ml under various magnetic field conditions for MNP pretreatments (NMF/NMF, CMF/NMF, or PMF/NMF). Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparison tests ($\alpha = 0.05$).

REFERENCES AND NOTES

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